172. (New) The oligomer of Claim 169, wherein said oligomer comprises from 2 to 50 monomer units of a sequence of said recombinant protein.

173. (New) The recombinant protein of Claim 151, which is conjugated to a carrier molecule.

174. (New) The recombinant protein of Claim 152, which is conjugated to a carver molecule.

175. (New) The recombinant protein of Claim 153, which is conjugated to a carrier molecule.

REMARKS

Claims 134-137, 139-143, 145, 148, 149 and 150-175 are active in the application. Claims 134, 136, 137, 140, 143, 145 and 149 have been amended for clarity. Claim 150 finds support in Claim 144. Claims 151-175 are supported by the disclosure on pages 16, 17 and 22 of the application as filed. No new matter has been added.

Applicants have now submitted a substitute Sequence Listing and a corresponding computer-readable Sequence Listing. Sequence Identifiers (SEQ ID NO:) have been added to the specification. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the substitute Sequence Listing. Support for all of the sequences listed in the substitute Sequence Listing is found in the present application as originally filed. In particular, Applicants note that SEQ ID NOS:7 and 8 corresponds to the sequence presented in Figure 1C and SEQ ID NO:15 is found on page 9, line 12. No new matter is believed to have been introduced by the submission of the substitute Sequence Listing and the corresponding computer-readable Sequence Listing.

The specification has been amended to correct the identification of drawings labeled

as Figure 12, to correct the reference on page 4, and to correct typographical errors page 35.

The objection to the disclosure under 35 U.S.C. § 132 is obviated by the attached substitute Sequence Listing.

The rejection of Claims 117 to 149 under 35 U.S.C. § 112, first paragraph ("written description") is obviated, in part, and respectfully traversed, in part.

Claims 117 to 133, 138, 144, 146 and 147 have been canceled.

The Examiner has taken the position that since the specification only discloses a few proteins, the description does not provide sufficient written description for the full scope of the claimed invention (see pages 6-7 of the Official Action). Applicants disagree.

The Examiner cites, for example, *The Reagents of the University of California v. Eli Lily* (43 USPQ 2d 1398) ("Lily") in support of his position. However, the facts of Lily and the present application are not the same. For the Examiner's reference, a copy of the cited case is attached. The question in Lily and the patent at issue, was whether there was a supporting description for any proinsulin DNA sequence, when the specification only provided the sequence of the rat proinsulin cDNA. In particular, the court stated: "a description of rat insuling cDNA is not a description of the broad classes of vertebrate or mammalian insulin cDNA." *Id* at 1568.

Unlike the situation in Lily, the present claims are directed to C-terminal fragments of a surface protein 1 of a merozoite form (MSP-1) from *Plasmodium* and unlike the situation in Lily, various MSP-1 proteins from a variety of *Plasmodium* organisms were known in the art at the time of filing the application. This is disclosed in the specification on pages 1-2 where the Applicants have referenced several published scientific articles that described the MSP-1 sequences. In addition, several of the prior art references cited by the office makes it clear

that the MSP-1 sequences were known, for example, see Shi et al, Eagan et al, Holm et al, Chang et al, Druilhe et al,and Holder et al, all of which are of record int he present application. Furthermore, the specification provides several examples of these known MSP-1 sequences in the Figures, for example, see Figure 4 and Figure 1.

According to the Guidelines for examination of patent applications under 35 U.S.C. § 112, first paragraph for the written description requirement (January 5, 2001), require that in issuing a rejection for lack of written description, a review of the entire application is required and:

> Such a review is conducted from the standpoint of one of skill in the art at the time the application was filed and should include a determination of the field of the invention and the level of skill and knowledge in the art. (Guidelines, II 2.)

The Examiner has not provided a detailed description as to why the skilled artisan would not be able to envision the full scope of the claims provided with the present disclosure and the common knowledge in the art. Applicants submit that in view of this description and knowledge, the skilled artisan can, in fact, envision the full scope of the claimed invention and as such the written description requirement under 35 U.S.C. § 112, first paragraph is met.

Withdrawal of this rejection is respectfully requested.

The rejection of Claims 119, 136 and 149 under 35 U.S.C. § 112, first paragraph is obviated, in part, and respectfully traversed, part.

Claim 119 has been canceled.

The Examiner has taken the position that the claims should be limited to those of *P. vivax* in view of Figures 12.1 A to 12.1 C and Annex II, which describe the NMR fingerprints

for only *P. vivax* (see page 7 of the Official Action). Applicants disagree.

The present specification also provides Annex I and Annnex III, which describe the atomic coordinates for *P. falciparum* and *P. cynomogoli.* Applicants also direct the Examiner's attention to page 40, last paragraph, which describes these Annexes.

Figures 12.2a to 12.2c, which describe the NMR fingerprints for *P. falciparum*, and Figures 12.02 to 12.0c, which describe the NMR fingerprints for *P. cynomolgi.*

Withdrawal of this rejection is respectfully requested.

The rejection of Claims 117 to 149 under 35 U.S.C. § 112, second paragraph is obviated, in part, and respectfully traversed, in part.

Claims 117 to 133, 138, 146 and 147 have been canceled.

The claims have been amended to have antecedent basis for the "surface protein," "merozoite form," "surface" and "the end of its penetration phase" as recited in Claims 134, and 145. Claim 136 has been amended to provide antecedent basis for the terms "atomic coordinates" and "NMR fingerprints. Claim 140 has been amended such that there is antecedent basis for C -terminal, "cleavage and "same MSP-1" protein. Claim 143 has been amended such that antecedent basis now exists for "said essential constituent polypeptide," "membrane" and MSP-1 protein." Claim 149 has been amended to refer to Claim 145 and antecedent basis now exists for "atomic coordinates" and "NMR fingerprinting."

As far as the relationship of the conformational epitopes to the two epidermal growth factor regions, these claims have been amended to clarify this relationship.

The phrase "human antisera" is not indefinite. "Antisera" is a known term meaning "human or animal serum containing antibodies for at least one antigen" (see the enclosed definition of "antiserum" from the American Heritage Dictionary of the English Language,

Houghton Mifflin Company, Boston, MA (1981).

Claim 137 limits Claim 134 to the vaccinating composition which elicits a long term memory response against the conformational epitopes. A long term memory response is in contrast to a short term memory response or an intermediate memory response. For example, see the attached discussion of Immunological Memory on pages 1323-1325 from "Fundamental Immunology", Third Edition, William E. Paul (Ed.), Raven Press, New York (1993).

Claim 139 has been amended to recite that the vaccinating composition further comprises the C-terminal region of p33 upstream of the 19 kilodalton C-terminal fragment. Applicants direct the Examiner's attention to page 9 of the specification and Figure 4, which clearly define this region.

Claim 143 has been amended and defines a glycosylphosphatidylinositol group which provides a function to the p19 protein.

Claim 144 now depends on Claim 145.

Conjugation as it relates to Claim 148 relates to the composition as defined in the claim. Conjugation of a vaccinating composition or antigen is well-known in the art as evidenced by the attached page 1316 from "Fundamental Immunology", Third Edition, William E. Paul (Ed.), Raven Press, New York (1993).

Withdrawal of this rejection is respectfully requested.

The rejection of Claims 117 to 128, 133 and 149 under 35 U.S.C. § 102(b) over Egan et al. (1995) is obviated by amendment.

Claims 117-128 and 133 have been canceled. Claim 149 now depends from Claim 145.

Egan et al do not anticipate the pending claims for the following reasons.

Egan et al disclose the relationship between cellular and humoral immune responses to defined epitopes of the C-terminal MSP-1 protein of *Plasmodium falciparum* in immune blood donors. Egan et al further disclose recombinant constructs derived from the Wellcome isolates and the MAD20 isolates of *P. falciparum* using the EGF motifs from these isolates. The constructs shown in Figure 1 have 96 amino acids. However, Egan et al fail to teach a vaccinating composition with alum (see Claim 134 and dependent Claim 149).

Egan et al also fail to disclose a recombinant protein having a leader sequence from *P. vivax* and a 19 kilodalton C-terminal fragment of a surface protein 1 of a merozoite form (a MSP-1 protein) *of Plasmodium falcipanim* from Asn_{1613} to Ser_{1705} or Asn_{1613} to Ser_{1726} or a portion of said C-terminal fragment which induces an immune response which can inhibit parasitemia *in vivo* in a host infected with a *Plasmodium* parasite (see Claims 151-175).

Withdrawal of this rejection is respectfully requested.

The rejection of Claims 119 and 149 under 35 U.S.C. § 102(b) over Shi et al, as evidenced by Egan et al is obviated by amendment.

Claim 119 has been canceled. Claim 149 now depends from Claim 145.

Shi et al do not anticipate the pending claims for the following reasons.

Shi et al relates to three known natural variant forms of the yeast-expressed recombinant 19-kDA fragment of *Plasmodium falciparum* that are referred to as E-KNG, Q-KNG and E-TSR antigens. These variants have either a Q or E in the first EGF-like domain at position 1644 and in the second EGF-like domain, amino acid residues at positions 1691, 1700 or 1701 have been found to be either TSR or KNG. Shi et al does not describe the MSP-1 protein, but 2 unique recombinant peptides of MSP-1 and 20 other non-MSP-1

peptides.

Shi et al fail to teach a vaccinating composition with alum (see Claim 134 and dependent Claim 149).

Shi et al also do not disclose a recombinant protein having a leader sequence from *P. vivax* and a 19 kilodalton C-terminal fragment of a surface protein 1 of a merozoite form (a MSP-1 protein) *of Plasmodium falciparum* from Asn_{1613} to Ser_{1705} or Asn_{1613} to Ser_{1726} or a portion of said C-terminal fragment which induces an immune response which can inhibit parasitemia *in vivo* in a host infected with a *Plasmodium* parasite (see Claims 151-175).

Moreover, Shi et al do not disclose any another *Plasmodium* specie other than *falciparum,* such as *Plasmodium cynomolgi.* Therefore, the Examiner's allegation that the Shi et al disclosure inherently provides the atomic coordinates and NMR fingerprints as in the present claims is misplaced because the proteins in Shi et al are not the same as those claimed.

Withdrawal of this rejection is respectfully requested.

The rejection of Claims 119 and 149 under 35 U.S.C. § 102(b) over Egan et al (1997) is obviated by amendment.

Claim 119 has been canceled. Claim 149 has been amended to depend on Claim 145. Egan et al do not anticipate the pending claims for the following reasons.

Egan et al fail to disclose a vaccine composition having alum (see Claim 134 and dependent Claim 149).

Egan et al produced recombinant proteins using 80 amino acids from the EGF-like domains of $MSP\text{-}1_{19}$ as a fusion protein from *Plasmodium cynomolgi* with S-transferase. However, Egan et al do not disclose a recombinant protein having a leader sequence from *P.*

vivax and a 19 kilodalton C-terminal fragment of a surface protein 1 of a merozoite form (a MSP-1 protein) *of Plasmodium falciparum* from Asn_{1613} to Ser_{1705} or Asn_{1613} to Ser_{1726} or a portion of said C-terminal fragment which induces an immune response which can inhibit parasitemia *in vivo* in a host infected with a *Plasmodium* parasite (see Claims 151-175).

Withdrawal of this rejection is respectfully requested.

The rejection of Claims 117-128, 133 and 149 under 35 U.S.C. § 102(a) over Chang et al. Is obviated by amendment.

Claims 117 to 128 and 133 have been canceled. Claim 149 now depends from Claim 145. Chang et al do not anticipate the pending claims for the following reasons.

Chang et al fail to disclose a vaccine composition having alum (see Claim 134 and dependent Claim 149).

Chang et al disclose a $rMSP\text{-}1_{19}$ construct corresponding to the FUP MSP-1 coding region from Asn_{1613} to Ser_{1705}. This recombinant construct was expressed as a fusion protein with the pre-pro-sequence of yeast alpha factor and a C terminal six histidine tag.

Chang et al also do not disclose a recombinant protein having a leader sequence from *P. vivax* and a 19 kilodalton C-terminal fragment of a surface protein 1 of a merozoite form (a MSP-1 protein) *of Plasmodium falciparum* from Asn_{1613} to Ser_{1705} or Asn_{1613} to Ser_{1726} or a portion of said C-terminal fragment which induces an immune response which can inhibit parasitemia *in vivo* in a host infected with a *Plasmodium* parasite (see Claims 151-175).

Withdrawal of this rejection is respectfully requested.

The rejection of Claims 117-120, 126 to 128, 131 and 132 under 35 U.S.C. § 102(b)

over Murphy et al, in light of Blackman et al is obviated by amendment.

Claims 117-120, 126 to 128, 131 and 132 have been canceled.

Murphy et al do not anticipate the pending claims for the following reasons.

Murphy et al disclose recombinant proteins containing the signal peptide of the precursor to the major merozoite surface antigen fused to a fragment from the carboxy terminus of the same gene. As shown in Figure 2 in Murphy, these recombinant proteins contained 293 amino acids from the major merzoite surface antigens. Transfer vectors pS42ΔA and $pSC_{26}42\Delta A$ were constructed by the deletion of the putative anchor sequence to obtain secretion of these hybrid proteins. The sequences for all of the recombinant constructs were derived from *P. falciparum.*

Murphy et al do not disclose a recombinant protein having a leader sequence from *P. vivax* and a 19 kilodalton C-terminal fragment of a surface protein 1 of a merozoite form (a MSP-1 protein) *of Plasmodium falciparum* from Asn_{1613} to Ser_{1705} or Asn_{1613} to Ser_{1726} or a portion of said C-terminal fragment which induces an immune response which can inhibit parasitemia *in vivo* in a host infected with a *Plasmodium* parasite (see Claims 151-175).

Moreover, Murphy et al fail to disclose use of any another *Plasmodium* specie, such as *Plasmodium cynomolgi.*

Withdrawal of this rejection is respectfully requested.

The rejection of Claims 117-120, 128, 131 and 132 under 35 U.S.C. § 102(b) over Blackman et al is obviated by amendment.

Claims 117-120, 128, 131 and 132 have been canceled.

Blackman et al do not anticipate the pending claims for the following reasons.

Blackman et al disclose a recombinant construct, s42ΔA which when expressed

produces a soluble excreted protein corresponding to the final 291 amino acid residues of the C-terminus of the MSP1 of the Wellcome *P. falciparum* isolate, without the hydrophobic anchor region. The MSP1 sequence used in the construct disclosed in Figure 1 extends from Asp_{1432} to Cys_{1722}.

However, Blackman et al do not disclose a recombinant protein having a leader sequence from *P. vivax* and a 19 kilodalton C-terminal fragment of a surface protein 1 of a merozoite form (a MSP-1 protein) *of Plasmodium falciparum* from Asn_{1613} to Ser_{1705} or Asn_{1613} to Ser_{1726} or a portion of said C-terminal fragment which induces an immune response which can inhibit parasitemia *in vivo* in a host infected with a *Plasmodium* parasite (see Claims 151-175).

Moreover, Blackman et al fail to disclose any another *Plasmodium* specie, such as *Plasmodium cynomolgi*.

Withdrawal of this rejection is respectfully requested.

The rejection of Claims 134 to 136, 138 to 142, 144 and 148 under 35 U.S.C. § 102(b) Burghaus et al is respectfully traversed.

The Examiner has not afforded these claims benefit of the priority document because "the subject matter has not been accorded the benefit of the French priority document because, as seen in the certified translation thereof provided by applicant, this document is silent on vaccinating compositions comprising alum." (See page 15 of the Office Action). Applicants disagree.

Applicants direct the Examiner attention to page 7, second to last paragraph through page 8 of the priority document:

> The aim of the present invention is to produce vaccinating recombinant proteins which can escape

these difficulties, for which protective effect 19 is verifiable in genuinely significant experimental models or even directly in man.

More particularly, the invention provides vaccinating compositions against a *Plasmodium* type parasite which is infectious in man, containing as an active principle a recombinant protein which may or may not be glycosylated, whose essential constituent polypeptide sequence is:

- either that of a 19 kilodalton (p19) C-terminal fragment of the surface protein 1 of the merozoite form (MSP-1 protein) of a Plasmodium type parasite which is infectious for man, said C-terminal fragment remaining normally anchored to the parasite surface at the end of its penetration phase into human erythrocytes in the event of an infectious cycle;

- or that of a portion of that fragment which is also capable of inducing an immune response which can inhibit *in vivo* parasitemia due to the corresponding parasite;

- or that of an immunologically equivalent peptide of said p19 fragment or said portion of that fragment; and said recombinant possibly protein further comprising conformational epitopes which are unstable in a reducing medium and which preferably constitute the majority of the epitopes recognised by human antiserums formed against the corresponding *Plasmodium*.

In addition, on pages 23 through 24 of the French priority document discloses a vaccination test with a recombinant *Plasmodium falciparum* p19 in the squirrel monkey in which 4 animals were injected with 50 pg of soluble PfMSPp19 in the presence of 10 mg of alum. See also Figure 7 and the description at page 24 where alum is described.

Thus, Applicants request that benefit of the French priority date be provided to the Claims 134 to 136, 138 to 142, 144 and 148.

Withdrawal of this rejection is respectfully requested.

The rejection of Claims 117 to 127, 129, 130 and 149 under 35 U.S.C. § 103(a) over

Longacre (1995) in view of Longacre et al (1994) is obviated by amendment.

Claims 117 to 127, 129 and 130 have been canceled. Claim 149 now depends from Claim 145, a vaccinating composition. Claims 151-175 are not obvious over the combination of Longacre and Longacre et al for the following reasons.

Longacre (1995) disclose the homology of *Plasmodium cynomolgi* MSSP-1 protein C-terminal sequence with other Plasmodium species. In Figure 1 the homolgies between the 42- kilodalton and 19- kilodalton fragment is illustrated. This article suggests using *P. cynomolgi* as a natural host parasite system to test the potential of the C-terminal region of MSP-1 as a vaccine for *P. vivax* malaria. This reference does not teach the production of recombinant proteins.

Longacre et al (1994) disclose the production of *Plasmodium vivax* merozoite surface protein 1 C-terminal recombinant proteins in baculovirus. Knowledge of the *P. falciparum* cleavage sites for p42 and p19 were used to identify the probable cleavage sites for the *P. vivax* MSP-1 homologue. Longacre et al (1994) teaches using two different C-terminal fragments between Asp_{1325} and Leu_{1726} and Asp_{1325} to Leu_{1704}, which recombinant proteins had between 369 to 401 amino acids.

However, nothing in the combination of these two references suggests a recombinant protein having a leader sequence from *P. vivax* and a 19 kilodalton C-terminal fragment of a surface protein 1 of a merozoite form (a MSP-1 protein) *of Plasmodium falciparum* from Asn_{1613} to Ser_{1705} or Asn_{1613} to Ser_{1726} or a portion of said C-terminal fragment which induces an immune response which can inhibit parasitemia *in vivo* in a host infected with a *Plasmodium* parasite (see Claims 151-175). In Claims 151-175, the maximum number of amino acids used in the recombinant constructs is from about 92 amino acids to about 113 amino acids.

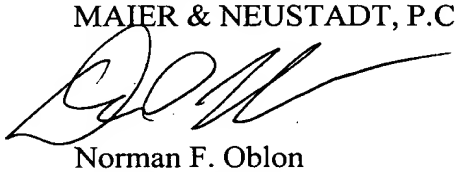
There is simply no suggestion in the references to change the teachings of the Longeacre disclosures to arrive at the claimed invention.

Withdrawal of this rejection is respectfully requested.

In view of the above, the present application is now in a condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



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IN THE SPECIFICATION

Page 4, please delete the first full paragraph and replace it with the following new paragraph:

--In any event, what the real vaccination rate would be which could possibly be obtained with such recombinant proteins is also questionable, bearing in mind the discovery- reported below- of the presence in p42s from *Plasmodiums* of the same species, and more particularly in the corresponding p33s of hypervariable regions which would in many cases render uncertain the immunoprotective efficacy of antibodies induced in individuals vaccinated with *a* p42 from a *Plasmodium strain* against an infection by other strains of the same species [(13)] (14).--

Page 4, after line 15, replace the text entered in the amendment on July 24, 2000 with the following text:

--BRIEF DESCRIPTION OF THE DRAWING--

Fig. 1A illustrates the nucleotide and amino acid sequences of the synthetic gene (Bac 19) and the "native gene" (PF19) of *P. falciparum* described by Chang et al.

Fig. 1B illustrates the nucleotide and amino acid sequences of the synthetic gene (Bac 19) and the "native gene" (PF19) of the Uganda Palo Alto isolate of *P. falciparum.*

Fig 1C illustrates the PfMSP1$_{P19}$A recombinant protein sequence before cutting out the signal.

Fig. 1D illustrates the PfMSP1$_{P19}$A recombinant protein after cutting out the signal

sequence.

Fig. 2A is an immunoblot using SDS-PAGE of the soluble recombinant $PfMSP1_{P19}A$ antigen purified by immunoaffinity in the presence (reduced) or absence (non-reduced) of β-mercaptoethanol.

Fig. 2B is an immunoblot with human antiserum of recombinant purified MSP-1 P19 from *P. vivax* and *P. cynomolgi* under non-reduced (NR), reduced only in the charging medium (R) and irreversibly reduced (IR) conditions.

Fig. 3A is an immunoblot of the soluble $PvMSPl_{p42}$ recombinant antigen in the presence of protein fractions derived from merzoites of *P. falciparum* and separately isoelectric focusing in the presence (reduced) or absence (nonreduced) of β-mercaptoethanol.

Fig. 3B is a graph illustrating the results of an ELISA inhibition technique of *P. vivax* MSP-1 P42 and P19 antigens by the antiserum of individuals with an acquired immunity to *P. vivax*.

Fig. 4 recites nucleotide sequences. The underlined oligonucleotides originate from *P. vivax* and are used as primers in a PCR reaction. The lower portion of Fig.4 illustrates the percent identity between two isolates of *P. vivax* and *P. cynomolgi*.

Fig. 5 shows curves illustrating the variation in the measured parasitemia as the number of parasited red blood cells per microliter of blood as the function of time passed after infection. Curve A corresponds to the average values observed in three vaccinated monkeys and curve B corresponds to the average values in five controls.

Fig. 6A is a graph illustrating the parasitemia observed in non-vaccinated control animals as a function of time after injection.

Fig. 6BA is a graph illustrating the parasitemia observed in control animals which contained a saline solution also contain Freunds adjuvant as a function of time after injection.

Fig. 6C is a superposition of Figures 6A and 6B.

Fig. 6D is a graph illustrating parasitemia at the end of vaccination with p42 as a function of time.

Fig. 6E is a graph illustrating parasitemia in animals vaccinated with pl9 alone as a function of time.

Fig. 6F is a graph illustrating parasitemia in animals with a mixture of P42 and P19 as a function of time.

Fig. 6G is the data obtained to produce the graphs in Figs. 6A to 6F.

Fig. 7A is an immunoblot illustrating the in vivo response of monkeys to injections of pl9 with Freunds adjuvant (1), with alum (2) and in the form of liposomes (3).

Fig. 7B is an immunoblot illustrating the in vivo response of a squirrel monkey after three injections with p19 with Freunds adjuvant, with alum and in the form of liposomes.

Fig. 8A is a graph illustrating the percent parasitemia versus days post infection of six monkeys, which were immunized with recombinant MSP-1 (pl9) six months earlier.

Fig. 8B is a graph illustrating the percent parasitemia versus days post infection of six monkeys that were immunized with normal saline and an adjuvant.

Fig. 8C is a graph illustrating the percent parasitemia versus days post infection of monkeys that were used as controls.

Fig. 8D is the data obtained to produce the graphs in Figs 8A to 8C.

Fig. 9A is a graph illustrating the percent parasitemia versus days post infection of 2 macaques immunized with recombinant pl9 and alum.

Fig. 9B is a graph illustrating the percent parasitemia versus days post infection of 2 macaques immunized with recombinant pl9 and alum.

Fig. 9C is a graph illustrating the percent parasitemia versus days post infection of a macaque immunized with pl9.

Fig. 9D is a graph illustrating the percent parasitemia versus days post infection of 3

control macaques immunized with physiological water and alum.

Fig. 9E is the data obtained to generate the graphs in Figs 9A to 9D.

Fig. 10A is a graph illustrating the percent parasitemia versus days post infection in a squirrel monkey immunized with MSP-1 p19 and alum.

Fig. 10B is a graph illustrating the percent parasitemia versus days post infection in a squirrel monkey immunized with MSP-1 p19 and Freunds.

Fig. 10C is a graph illustrating the percent parasitemia versus days post infection in a squirrel monkey immunized with MSP-1 p19 with liposomes.

Fig. 10D is a graph illustrating the percent parasitemia versus days post infection in a squirrel monkey immunized with alum as the control.

Fig. 10E is a graph illustrating the percent parasitemia versus days post infection in a squirrel monkey immunized with Freunds as the control.

Fig. 10F is a graph illustrating the percent parasitemia versus days post infection in a squirrel monkey immunized with liposomes as the control.

Fig. 10G is a graph illustrating the percent parasitemia versus days post infection in a squirrel monkey immunized with physiological water as the control.

Fig. 11A is a drawing of the backbone of $MSP1_{19}$ from *P. cynomolgi* showing disulfide bridges in bold line.

Fig. 11B is a drawing of the backbone of $MSP1_{19}$ showing positions of sequence differences between P. *cynomolgi and P. vivax.*

Fig. 11C is a drawing of the backbone of homology-modeled $MSP1_{19}$ of *P. falciparum* showing positions of sequence differences with *P. cynomolgi.*

Fig. 12 D is a NOESY spectrum of *P. vivax* $MSP1_{19}$.

Fig. 12 E is a NOESY spectrum of *P. vivax* $MSP1_{19}$.

Fig. 12 F is a NOESY spectrum of *P. vivax* $MSP1_{19}$.

Fig. 12.0a is a NOESY spectrum *of P.cynomolgi* $MSP1_{19}$.

Fig. 12.0b is a NOESY spectrum of *P. cynomolgi* $MSP1_{19}$.

Fig. 12.0c is a TOCSY spectrum of *P. cynomolgi* $MSP1_{19}$.

Fig. 12.1a is a NOESY spectrum of *P.vivax* $MSP1_{19}$.

Fig. 12.1b is a NOESY spectrum of *P. vivax* $MSP1_{19}$.

Fig. 12.1c is a TOCSY spectrum of *P. vivax* $MSP1_{19}$.

Fig. 12.2a is a NOESY spectrum of *P. falciparum* $MSP1_{19}$.

Fig. 12.2b is a NOESY spectrum of *P. falciparum* $MSP1_{19}$.

Fig. 12.2c is a TOCSY spectrum of *P. falciparum* $MSP1_{19}$.

[Fig. 12A is a NOESY spectrum of *P. cynomolgi* $MSP1_{19}$.

Fig. 12B is a NOESY spectrum of *P. cynomolgi* $MSP1_{19}$.

Fig. 12C is a TOCSY spectrum of *P. cynomolgi* $MSP1_{19}$.

Fig. 12.1A is a NOESY spectrum of *P. vivax* $MSP1_{19}$.

Fig. 12.1B is a NOESY spectrum of *P. vivax* $MSP1_{19}$.

Fig. 12.1C is a TOCSY spectrum of *P. vivax* $MSP1_{19}$.

Fig. 12.2A is a NOESY spectrum of *P. falciparum* $MSP1_{19}$.

Fig. 12.2B is a NOESY spectrum of *P. falciparum* $MSP1_{19}$

Fig. 12.2C is a TOCSY spectrum of *P. falciparum* $MSP1_{19}$.]

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Page 8, line 21 to page 9, line 12, replace the text in its entirety with the following:

The 19 kDa C-terminal fragment, the sequence of which is present in the active principle of the vaccine, can be limited to the sequence for the p19 itself, in the absence of any polypeptide sequence normally upstream of the p19 sequence in the corresponding MSP-1 protein. Clearly, though, the essential constiuent polypeptide sequence for the C-terminal

side belonging to the 33 kDa (p33) N-terminal fragment still associated with the p19 in the corresponding p42, before natural cleavage of the latter, if the presence of this fragment does not modify the immunological properties of the active principle of the vaccine. As will be seen below, in particular in the description of the examples, the C-terminal sequences of the p33 in various strains of the same species of *Plasmodium* (see the C-terminal portion of the peptide sequences of "region III" in Figure 4 (SEQ ID NOS:11-14)) also have a degree of homology or substantial conservation of the sequence, for example on the order of at least 80%, in different varieties of *Plasmodiums* which are infectious for man, such that they do not fundamentally modify the vaccinating properties of the active principle (the sequence of which corresponds to region IV in Figure 4 (SEQ ID NOS:11-14)), in particular using the hypothesis which follows from this figure; that the presumed cleavage site between the p19 and region III of the p33 is located between the leucine and asparagines residues in a particularly well conserved region (LNVQTQ- SEQID NO:15).

Page 21, line 19 page 22, line 6, replace the text in its entirety with the following:

A 1200 base pair fragment was produced using a PCR reaction using the oligonucleotides underlined in **Figure 4** originating from *P. vivax* (see amino acids 1-6 and 373-380 of SEQ ID NO:13). The 5' oligonucleotide comprised an EcoRI restriction site and the 3' oligonucleotide comprises two synthetic TAA stop codons followed by a BglII restriction site. This fragment was introduced by ligation and via these EcoRI and BglII sites into the $pVLLSV_{200}$ plasmid already containing the signal sequence for the MSP-1 protein of *P. vivax* (19). The new plasmid ($pVLSV_{200}C_{42}$) was used to analyze the DNA sequences.

The *P cynomolgi* (SEQ ID NO:11) and the corresponding *P. vivax* (SEQ ID NOS:12 and 13) sequences were aligned. The black arrows designate the presumed primary and secondary cleavage sites. They were determined by analogy with known sites in

P.falciparum (27, 28). The vertical lines and horizontal arrows localize the limits of the four regions which were studied. Region 4 corresponded to the sequence coding for the *P. cynomolgi* p19. Glycosylation sites are boxed and the preserved cysteines are underlined. The lower portion of **Figure 4** shows the percentage identify between the two isolates of *P. vivax* and *P.cynomolgi.*

Page 33, lines 12-16, replace the text in its entirety with the following:

In the present case, the most hypervariable regions are defined as region II or region II and all or part of region III, the portion of region II which is preferably deleted being that which is juxtaposed to region II (the conserved portion being located to the side of the C-terminal of p33, close to the p19). Regions II and III are illustrated in Figure 4 (SEQ ID NOS: 11-14).

Page 35, lines 6-19 replace the text in its entirety with the following:

--The invention relates also particularly to recombinant proteins, as obtainable in a baculovirus vector system:

-in a pure state

-substantially free of any other form of recombinant protein which, has the same peptide sequences, but which contains alternate conformations in the two EGF regions. This alternate conformation is different from the conformational form as defined by:

-(a) the atomic coordinates as defined in Annexes I, II or [II] III obtained by crystallography (the Annexes 1, II or [II] III include respectively the atomic coordinates which define the *P. cynomolgi* $MSP1_{19}$, *P. vivax* $MSP1_{19}$ and *P. falciparum* $MSP1_{19}$ three-dimensional molecular structure); and

-(b) the NMR fingerprints as illustrated in Figures 12.0a to 12.2c.--

After the last page (Abstract), replace the Sequence Listing filed October 22, 1999 with the substitute Sequence Listing attached hereto.

IN THE CLAIMS

Please amend the claims as follows:

134. (Amended) A vaccinating composition against a *Plasmodium* parasite which is infectious in man, comprising as an active principle a recombinant protein whose essential constituent polypeptide sequence comprises:

a) a 19 kilodalton (p19) C-terminal fragment of [the] a surface protein 1 of [the] a merozoite form (MSP-1 protein) of a *Plasmodium* parasite that is infectious in man, other than *Plasmodium vivax[,* wherein said C-terminal fragment remains anchored to the surface of said *Plasmodium* parasite at the end of its penetration phase into human erythrocytes during an infectious cycle]; or a portion of said 19 kilodalton (p19) [C-Terminal] C-terminal fragment, other than a fragment from *Plasmodium vivax,* which induces an immune response and which can inhibit parasitemia *in vivo* in a host infected with said *Plasmodium* parasite; wherein said C-terminal fragment remains anchored to the surface of said plasmodium parasite at [the] an end of its penetration phase into human erythrocytes during an infectious cycle and wherein said recombinant protein comprises conformational epitopes recognized by human antisera[, contains] which are contained in two epidermal growth factor regions and is unstable in a reducing agent; and

b) alum.

136. (Amended) The vaccinating composition of Claim 134, wherein said 19 kilodalton (p 19) C-terminal fragment of the surface protein 1 of the merozoite form (MSP-1 protein) has [the] atomic coordinates in Annexes I[, II] or III; and [the] NMR fingerprints of

Figures 12.0a to [12.2c] 12.0c or 12.2a to 12.2c.

140. (Amended) The vaccinating composition of Claim 139, wherein said polypeptide region is the C-terminal region of p33 resulting from the cleavage of p42 of [the] a same MSP-1 protein.

143. (Amended) The vaccinating composition of Claim 134, wherein said [polypeptide has] C-terminal fragment remains anchored to the surface of said *Plasmodium* parasite via a glycosylphosphatidylinositol group which anchors the pl9 fragment to the membrane of a eukaryotic cell infected with the MSP-1 protein.

145. (Amended) A vaccinating composition against a *Plasmodium* parasite which is infectious in man, comprising as an active principle a recombinant protein whose essential constituent polypeptide sequence comprises:

a) a 19 kilodalton (pl9) C-terminal fragment of [the] a surface protein 1 of [the] a merozoite form (MSP-1 protein) of a *Plasmodium cynomolgi* parasite that is infectious in man, and wherein said recombinant protein comprises conformational epitopes recognized by human antisera, [contains] which are contained in two epidermal growth factor regions and is unstable in a reducing agent; and

b) alum.

149. (Amended) The vaccinating composition of Claim 145, wherein said 19 kilodaltan (p 19) C-terminal fragment of the surface protein 1 of the merozoite form (MSP-1) protein a) has [the] atomic coordinates in [Annexes I, II or III] Annex I; and the NMR fingerprints of Figures 12.0a to [1.2c] 12.0c.